1H-NMR and LC/MS assay development for the characterization of glycosidase and glycosyl transferase activities of MalA from Bdellovibrio bacteriovorus

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**Introduction**

Sequencing of the predatory bacteria *Bdellovibrio bacteriovorus* genome in 2001 revealed three potential carbohydrate-active genes, including a putative maltase MalA, which were unexpected given *Bdellovibrio's* observed disuse of prey carbohydrates. In order to understand MalA’s function in the life of the potentially useful living anti-biotic *Bdellovibrio*, the native substrate and activity of the enzyme must be determined. Determination of activity in glycolytic enzymes has traditionally been done using a glucose oxidase colorometric quantitation of glucose. This method is limited to determining the cleavage of glucose from longer oligosaccharides and does not allow for characterization of other possible enzymatic activities, including glycosyltransferase activity, which has been observed in MalA by qualitative TLC experiments.  

**1H-NMR method**

- **1H NMR was used to assess the relative concentrations of glucose and two maltoligosaccharides (α, 1-4 linked glucose polymers) by analyzing the anomeric region of 1H NMR spectra.**
- **The anomeric proton in maltoligosaccharides (and glycosyl radicals in DP > 1), as a hemiacetal (or glycosidic acetal), has a chemical shift significantly higher than any other proton. It appears with different shift depending on its anomeric configuration (Figure 2).**

**1H-NMR results**

The three studied sugars were found to be distinguishable by the β-anomer resonance (glucose from maltose or maltotriose), or the glycosidic proton resonance (maltose from maltotriose). The above method was applied to a continuous assay of MalA’s activity on glucose, maltose, and maltotriose, which has been observed in MalA by qualitative TLC experiments.

**LC/MS method**

- **All experiments were conducted on an Agilent 1100 series LC/MSD SL Ion Trap with an electrospray ionization (ESI) ion source.**
- **Pseudo-molecular ions [M+Cs+] were formed by addition of 40 μM cesium acetate in the 80/20 acetonitrile/water mobile phase.**
- The trap was optimized to maximize transmittance of the [Maltose+Cs+], pseudo-molecular ion (m/z = 475). Typically 5 μL of sample, dissolved in water or the mobile phase, was injected for each measurement. No chromatographic separation is currently in place.

**LC/MS results**

- **Pseudo-molecular ions [M+Cs+] were observed for glucose, maltose, and maltotriose (Figure 4).**

**Conclusions**

**1H-NMR studies on MalA**

- **1H-NMR is an efficient and powerful method for qualitatively screening glycosidase and glycosyltransferase activities on small carbohydrates.**
- **Glycosyltransfer activities in MalA on maltose and maltotriose, but not on trehalose (α-1→1) glucose dimer were observed by 1H-NMR.**
- **Maltotriose was seen to accumulate from maltose in the presence of MalA. This supports earlier observations of glycosyl transferase activities seen by TLC.**

**Quantitation of carbohydrates by LC/MS**

- **The LC/MS method has high potential for easily quantitating underivatized carbohydrates in solution, allowing for high-throughput screening of carbohydrate-active enzyme activities on various substrates.**
- **We should be able to precisely determine relative carbohydrate concentrations in solution in the range of roughly 1-1000 ng of each carbohydrate per 5 μL sample with a method time under 10 minutes per sample.**
- This means potentially detecting 0.1% or less changes in the carbohydrate composition of a solution, making detection by cesium attachment in LC/MS an ideal method for assaying carbohydrate-active enzymes.

**Future work**

- **Through method verification and optimization will be conducted for the three carbohydrates currently under study. We should be able to apply the same methodology to longer oligomers.**
- **Apply the method to determine relative concentrations of sugars in a mixture.**
- **Employing an amino or cyclodextrin stationary phase HPLC column for carbohydrate separation should allow for improved sensitivity. Properly separated samples should shine in shorter time periods, effectively concentrating ions for easier detection.**
- **Carbohydrates separated according to degree of polymerization, we can optimize ion transmission for each carbohydrate in solution, maximizing the sensitivity potential of the ion trap.**
- **Finally, separation will allow for fragmentation studies, which have been used in quantitation, and can provide structural information for unknown analytes.**
- **Continue organic synthesis toward small carbohydrate derivatives for kinetic studies with MalA, which will lead to insights on substrate-enzyme interactions.**

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**Literature cited**


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**For further information**

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